CHOLESTEROL METABOLISM STUDIED WITH FAT EMULSION

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by

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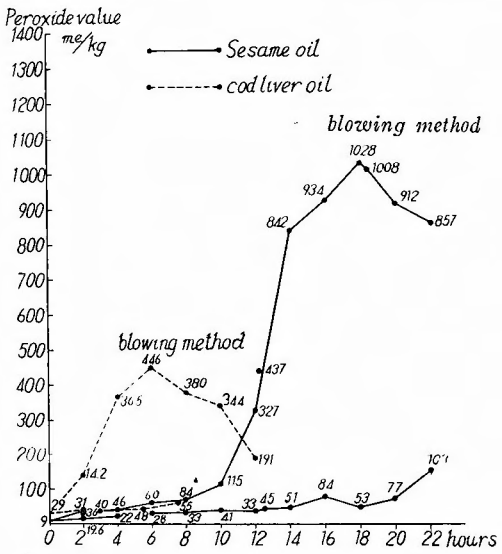
I. INTRODUCTION

It has been recognized that fat is one of the three main foodstuffs and has the highest caloric value. The caloric value of fat is more than twice that of carbohydrate or of protein. Fat is also important in the regulation of body temperature and for the fixation of organs by virtue of its presence in subcutaneous, perivisceral, intermuscular and mesenteric tissues.

It need scarcely be said that fat has a variety of important functions as variable elements; it serves as a caloric source to meet the energy demands of the body. It has another important function, which cannot be neglected; such fats as phospholipid, glycolipid and sterol play an indispensable role in the construction of tissues and the maintainance of their functions. Despite its importance as a nutrient, the parenteral administration of fat to patients has long been discouraged. Our laboratory has conducted a series of experiments to examine fat metabolism in vivo by means of intravenous administration of fat emulsion, and revealed the following facts. The mixed glycerides consisting of myristic, palmitic, stearic, oleic, linoleic and linolenic acid have some beneficial effects, while the other mixed glycerides containing a large quantity of lower fatty acids, highly unsaturated fatty acids and fatty acids having a single double bond and consisting of over 20 carbon atoms (docosenoic acid, eicosenoic acid etc.) are injurious to the body. Furthermore, care must be taken not to include any peroxide in the diet, because auto-oxidation frequently occurs during the cooking or preservation of fat and oil. It has been verified that the repeated intravenous administration of cod liver oil, containing highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc., mixed with some peroxides, always causes liver damage. In addition, KANEDA et al. have proved that the peroxides thus administered accumulate in the liver, muscles and other tissues and damage the mitochondria and eventually cause a serious disturbance in the enzyme system. KISHIMOTO in our laboratory has measured the peroxide value of various fats and shown that even sesame oil of J. P. IV. specification contains some peroxides, although sesame oil is quite free of lower fatty acids and highly unsaturated fatty acids (Fig. 1). Thus, it has been found that a certain amount of peroxides is introduced into the body in the daily diet. Therefore, sesame oil of peroxide value 0 has been used for preparing fat emulsions in our laboratory. However, this toxicity of peroxides has long been ignored by many investigators. Most of the experiments on the nutritional effects of

fat have been performed without proper respect to the qualitative properties of the fat. Therefore, we can hardly accept the results of these experiments. In the present experiments the author has, from the view-point of cholesterol metabolism, examined whether even fats with a low peroxide value (peroxide value.....5～10) might do any harm to the body and sought a preventive measure against it. At the same time we have studied the physiological significance of cholesterol by means of the intravenous administration of sesame oil emulsions with added cholesterol.

Fig. 1 Changes in peroxide value during blowing at 95°C and no blowing at the same temperature (by KISHIMOTO).



II. MATERIALS AND METHODS

A. MATERIALS

1) Fat Emulsion: In the present experiment 20% sesame oil emulsion containing glucose in a concentration of 7% and a small amount of a stabilizer was used. In addition, various mixtures of 20% sesame oil with added cholesterol in concentrations of 0.3%, 0.5%, 0.7% and 1.0% were prepared to be used in the different experiments.

2) Experimental Animals: A number of healthy rabbits weighing approximately 2.0 kg were used; they had been maintained on a fixed diet (bean-curd waste and vegetable) for a week so that they attained steady weight at the time of the experiments. Blood samples were regularly collected 24 hours after each feeding. Following HASHINO's method, 2.5 cc of the emulsion i. e. 0.5 g of glyceride per kg body weight was infused through the ear veins of the rabbits.

3) Clinical Cases: A number of patients were selected from those admitted to the 2nd Surgical Clinic of Kyoto University Hospital. They were drip-infused intravenously daily with 100 cc of the 20% sesame oil emulsion combined with various vitamins. This emulsion was diluted 1/10 in Ringer's solution for the first infusion and 1/5 from the second time on (Dilution in a 5% glucose solution or physiological saline solution is not adequate.). For the cases requiring surgery, 500 cc of a 5% glucose solution was added.

B. EXPERIMENTAL METHODS

1) Total Cholesterol (abbreviated as T-chol.): RAPPAPORT and ENGELBERG'S method was used. The setting of time and temperature of LIBERMAN-BURCHARD'S color reaction was based on the results of the preparatory experiment performed by the author (described below).

2) Cholesterol-ester (abbreviated as E-chol.): BLOOR and KNUDSON'S method

was used. The setting of time and temperature for the color reaction was based on the results of the preparatory experiment performed by the author (described below).

3) Phospholipid (abbreviated as PL): KIRK FLASK's method was used.

A SHIMAZU's electro-spectro-photometer was used in these measurements; T-chol. and E-chol. were measured at the wave-length of 640 mμ and PL at 660 mμ using a red filter.

4) Bromsulphalein Test (abbreviated as BSP): Hepatosulphalein of DAIICHI Pharmaceutical Co. was used and the measurements were made with 30 minute values (described below).

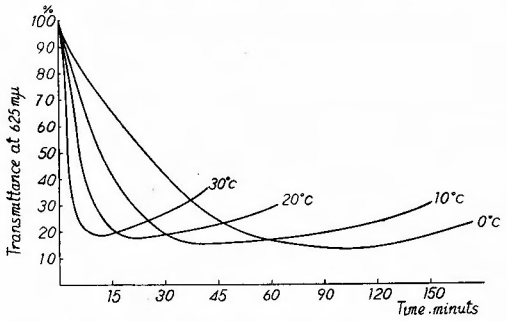
5) Cephalin-cholesterol Flocculation Test (abbreviated as CCFT): Cephalin-cholesterol flocculation antigen of SUMITOMO Chemical Co. was used.

6) Cholesterol-ester Ratio (abbreviated as EQ): This was expressed by the ratio of E-chol. to T-chol.

III. PREPARATORY EXPERIMENTS

The qualitative determination of T-chol. and E-chol. is achieved colorimetrically. Attention is called to the following facts. In the observation of the color reaction, changes in color are affected by the time and temperature at which the observation is made. The colors are as follows: blue, green-blue, green, green-yellow and yellow. Different methods adopt one color out of this series. The author examined and carefully evaluated each of the known methods and set the time and temperature at 10 minutes and 37°C respectively, in accordance with the experimental results of KINGSLEY et al. (Fig. 2). Because the color reaction progresses very quickly at 37°C, the sample was put into ice water immediately after the color reaction was obtained so as to prevent further progress of the reaction. It was measured with a SHIMAZU electro-spectro-photometer. The curve of optical density obtained under these conditions is shown in Fig. 3. The author set the quantitative determinations of T-chol. and E-chol. at the uniform wave-length of 640 mμ.

Fig. 2 Effect of environment temperature and time on color reaction of cholesterol (by KINGSLEY et al.).



IV. RESULTS AND DISCUSSION

A. RESULTS OF CLINICAL EXPERIMENTS

Cholesterol in the body has two sources: part is taken by mouth, the rest is synthesized within the body. It is known that an adult man takes 0.25 to 0.80 g of cholesterol by mouth. It is assumed that the esterification of cholesterol is necessary for its absorption, since cholesterol is esterized by pancreatic juices and it exists mainly in the esterized form in the thoracic duct lymph. Cholesterol thus

absorbed into the thoracic duct eventually enters the blood stream, and stays in plasma in the combined forms of α- and β-lipoprotein. In addition, cholesterol is synthesized by many tissues, but not by the brain, in the adult, totaling about 2.0 g daily. The liver is regarded as the principal producer of cholesterol, producing about 1.2 g of cholesterol daily. According to KEYS, the peroral introduction of cholesterol in the amount of 0.7 g daily has little effect on the serum cholesterol concentration, but the total calories of the diet has a direct relation to it, and the serum cholesterol concentration changes in parallel with the total amount of dietary fats, therefore, it is necessary not only to reduce the cholesterol intake but also to limit the dietary fats to less than one-quarter of the total caloric intake in order to normalize the serum cholesterol concentration.

As already mentioned in the introduction, the possibility always exists that the daily diet contains some peroxides as a result of auto-oxidation of fat. To determine whether such peroxides infused into the body can affect seriously the serum concentration of cholesterol, the following investigation was undertaken.

A 20% sesame oil emulsion containing cholesterol in a 0.5% concentration plus peroxides (peroxide value 5～10), and an emulsion containing the same concentration of cholesterol but free of peroxides were prepared. 100 cc of each of these emulsions

Fig. 3 Curve of optical density by RAPPAPORT & ENGELBERG's method.

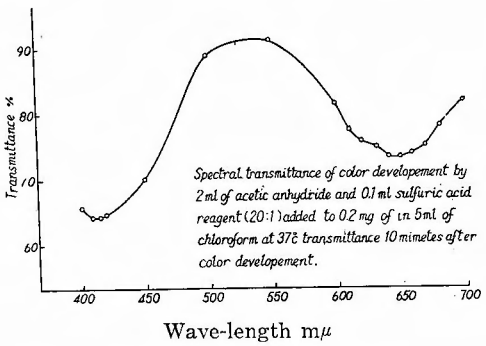
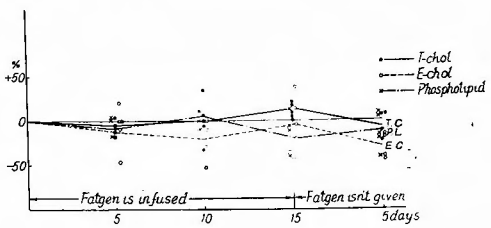


Fig. 4 Changes in blood cholesterol levels following repeated intravenous administration of sesame oil emulsion contaning cholesterol in concentration of 0.5% and free of peroxides (5 unoperated clinical cases).



was used with the addition of 20 mg of vitamin B_1, 20 mg of vitamin B_2 and 200 mg of vitamin C. The influence of the peroxides on cholesterol metabolism was examined by measuring the serum concentration of T-chol., E-chol., PL and EQ.

1) Serum Concentration of T-chol., E-chol. and EQ in Normal Adults

As shown in Table 2, the values of the above measurements are 187 ± 50 mg/dl, 132.6 ± 50 mg/dl and 70.9% respectively.

2) Changes in the Serum Concentration of T-chol., E-chol. and PL Observed Following the Infusion of 20% Sesame Oil Emulsion Containing Cholesterol in Concentration of 0.5% and Free of Peroxides

To 100 cc of this emulsion was added 20 mg of vitamin B_1, 20 mg of vitamin B_2 and 200 mg of vitamin C. This was diluted in RINGER's solution and infused intravenously into unoperated patients each day for 15 days (Table 3). As the

Table 2 Total cholesterol value and cholesterol ester value in normal adults. (Serum: mg per dl)

	Total cholesterol	Ester cholesterol
I. I. 송	258	182
T. U. 우	166	110
T. A. 송	184	84
O. K. 우	132	92
M. T. 우	152	135
H. O. 송	184	130
T. K. 송	182	126
K. K. 송	198	144
T. O. 우	184	177
B. U. 우	232	146
Average	187±50	133±50
		EQ 70.9%

Table 3 Unoperated cases treated with administration of sesame oil emulsion containing cholesterol in concentration of 0.5% and free of peroxides.

Patient	Age	Sex	Disease
A. K.	30	우	Esophageal stenosis
T. O.	29	송	Spontan gangrene
M. T.	23	우	Acroasphyxia
T. M.	22	송	Keloid
T. M.	32	우	Necrosis of abdominal wall

results of this experiment show in Fig. 4, no significant changes were observed in the serum concentration of T-chol., E-chol. and PL, and there was no disturbance of the esterification of cholesterol. It was revealed that the infused cholesterol was smoothly metabolized within the body. Further investigations were performed to observe the changes of serum T-chol., F-chol. and EQ when gastrectomized patients were infused.

100 cc of this emulsion with 20 mg of vitamin B_1, 20 mg of vitamin B_2 and 200 mg of vitamin C was diluted in 500 cc of RINGER'S solution and 500 cc of a 5% glucose solution and infused in gastrectomized patients daily. This procedure was repeated for 5 days prior to and 10 days after gastrectomy (Table 4). At the same

Table 4 Operated cases treated with administration of sesame oil emulsion containing cholesterol in concentration of 0.5% and free of peroxides.

Patient	Age	Sex	Disease	Operation
M. A.	38	송	Duodenal ulcer	Gastrectomy
J. H.	33	송	Gastric ulcer	Gastrectomy
T. U.	43	우	Gastric cancer	Gastrectomy
M. F.	33	송	Gastroptosis	Gastrectomy
T. S.	24	송	Gastric ulcer	Gastrectomy

Table 5 Operated cases treated with administration of 5% poltamin solution (control).

Patient	Age	Sex	Disease	Operation
K. I.	57	♂	Gastric cancer and hypertension	Ileosigmoidostomia
E. I.	44	♂	Gastric cancer	Gastrectomy
T. T.	42	♂	Gastric cancer	Total gastrectomy

Fig. 5 Changes in blood cholesterol levels following repeated intravenous administration of sesame oil emulsion containing cholesterol in concentration of 0.5% and free of peroxides (5 operated clinical cases).

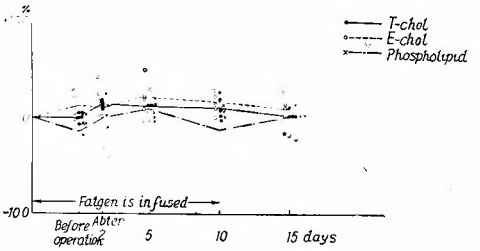
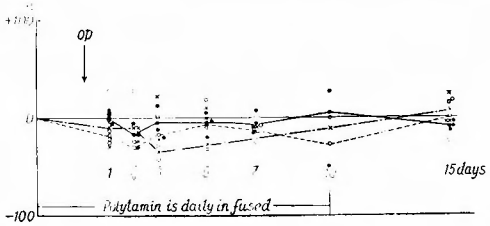


Fig. 6 Changes in blood cholesterol levels following repeated intravenous administration of 5% Polytamin solution as control (3 operated clinical cases).



time 100 cc of 5% polytamin solution was infused simultaneously with vitamins, a glucose solution and RINGER'S solution in the control group daily (Table 5). Then comparisons of serum T-chol., E-chol. and PL values were made between these two groups (Fig. 5, Fig. 6).

However, no significant difference was recognized between the two groups, nor any disturbance in the esterification of cholesterol, regardless of the liver function before the infusions. The slight changes in the values observed following operations in both groups could be attributed to such a stress as gastrectomy. These results show that repeated infusions of 0.5 g of cholesterol and 20 g of glyceride daily, even in gastrectomized patients, cause no serious disturbance in the cholesterol metabolism and the infused cholesterol was smoothly metabolized within the body. Simultaneous investigations of liver function were made by means of BSP and CCFT tests. Hepatosulphalein of DAIICHI Pharmaceutical Co. was used for the BSP test. INGELFINGER et al. has reported that this dye is excreted into the bile within 1 hour after intravenous injection and has no harmful side effects and it hardly affects renal function. According to MENDELOFF, it scarcely affects the reticuloendothelial system, eiter. TAKAHASHI applied it to the investigation of liver function in the cases of dermato-urological diseases. ISHIYAMA aslo applied it in surgical cases. Both of them have regarded it as an excellent test method for indications of operation and judgement of prognosis. In the present investigation, 5 mg of hepatosulphalein per kg was infused into a subcutaneous vein of one arm and blood was collected from a vein of the other side. Standard solutions of 2.5%, 5.0%, 7.5%, 10%...100% were prepared, and measured colorimetrically using a WALPOL comparatol. KURIHARA, OKUBO and KUWABARA define the normal value of BSP test as under 5% in 30

minutes and 0% in 45 minutes.

As for CCFT, it was performed by HANGER-KITANI's method using cephalin-cholesterol antigen of SUMITOMO Chemical Co., a refined extract of cattle brain. The normal value was defined at 18°C as under (+) for 24 hours, and under (++) for 48 hours. EQ was expressed by the ratio of E-chol. to T-chol. Because the liver plays the most important role in the esterification of cholesterol, EQ values are believed to reflect liver function to some extent. In this regard THANNHAUSER and SCHABER have called our attention to a remarkable fact that in severe hepatitis, especially in liver cirrhosis, not only T-chol. decreases but E-chol. also markedly falls sometimes even to zero, resulting in a severe drop of EQ, a condition called "Cholesterol-Ester Stürtz". In the present experiment a general examination was made by means of BSP, CCFT, EQ value and A/G ratio of serum etc., and the findings showed that the infusion of sesame oil emulsion did not cause any disturbance in liver function, nor did it aggravate the temporary disturbance of liver function due to operative procedures.

3) Changes in the Serum T-chol., E-chol. and PL Concentration Observed Following the Intravenous Infusion of 20% Sesame Oil Emulsion Containing Cholesterol in Concentration of 0.5% and Peroxides (Peroxide Value 5~10)

A daily total of 100 cc of sesame oil emulsion with various vitamins, glucose and RINGER's solution as described in the preceding experiments was infused intravenously. This procedure was repeated for 5 days prior to and 10 days after gastrectomy (Table 6).

Table 6 Operated cases treated with administration of sesame oil emulsion containing cholesterol in concentration of 0.5% and peroxides (Peroxide value: 5~10).

Patient	Age	Sex	Disease	Operation
M. Y.	46	우	Pyloric cancer	Gastrectomy
H. K.	31	송	Gastric ulcer	Gastrectomy
Z. Y.	41	송	Benign pylorus stenosis	Gastrectomy
T. F.	51	송	Duodenal ulcer	Gastrectomy
T. O.	54	송	Gastric ulcer	Gastrectomy
S. K.	27	송	Chronic gastritis	Gastrectomy

Examinations showed that though in some of the patients the same favorable results were attained as those infused with the sesame oil emulsion free of peroxides, in other patients, many of whom had some liver damage before the infusion, there was a marked rise in serum T-chol. concentration and a disturbance in the esterification of cholesterol (Fig. 7). A typical case is presented in Fig. 8. As these results demonstrate, the introduction of even a small amount of peroxides into patients suffering from some liver damage aggravates it and disturbs the cholesterol metabolizing function of the liver. In other words the infusions of peroxides, even of extremely low peroxide value (5~10), should by all means be avoided in pre- and postoperative patients, especially in those with some liver damage.

The proper administration of fat with an adequate understanding of its qualitative

Fig. 7 Changes in blood cholesterol levels following repeated intravenous administration of sesame oil emulsion containing cholesterol in concentration of 0.5% and peroxides (peroxide value: 5～10). (6 operated clinical cases)

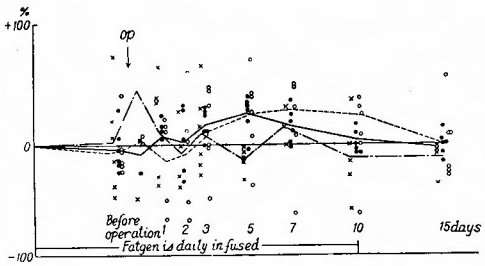
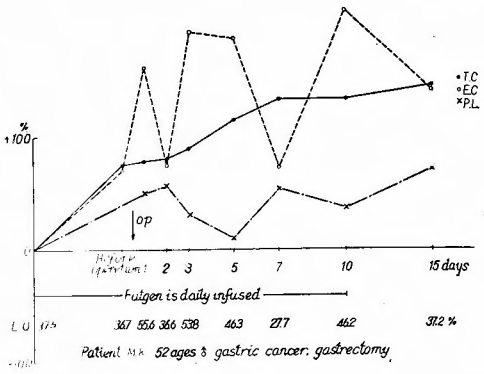


Fig. 8 A typical case showing significant rise of blood cholesterol level.



properties, namely the presence or absence of peroxides produced by auto-oxidation and the nature of the component fatty acids, exerts many favorable effects. For instance, it causes a marked protein sparing action as clarified by OSA, TSUKADA, HANAFUSA and KUYAMA of our laboratory, and maintains liver glycogen by means of its glycogenetic action as verified by MATSUDA of our laboratory. Moreover, NAGASE of our laboratory has clarified that fat, essential fatty acids particularly, plays a vital role in maintaining capillary resistance and controlling its permeability. Its shortage weakens capillary walls causing increased permeability, as the chief factor in capillary bleeding and edema.

From these facts it is clear that a fat supply is indispensable for complete nutrition, and that the prevailing misunderstandings, under which the administration of fat has been avoided during the pre- and postoperative state, must be immediately corrected and fat should be given positively even to surgical cases.

B. RESULTS OF ANIMAL EXPERIMENTS

1) Measures to Prevent Peroxide Toxicity

i) Serum Concentrations of T-chol., E-chol. and PL in Normal Rabbits

The values of these measured by the author are presented in Table 7 and Fig. 9. These show a wide range of normal values due to considerable individual variety.

Table 7 Total cholesterol, ester cholesterol and phospholipid value in blood (36 Rabbits)

	Total chol. (mg/dl)	Esterchol. (mg/dl)	Phospholipid (mg/dl)	E. Q (%)
Distribution	25～188	23～138	217～415	37.1～91.7
Average	107	68	300	68.23
Deviation	±81.5	±57.5	±99	±28

Therefore, it is necessary to determine the normal values for each group before a comparison of the amount of the constituents of serum fat can be made. In the following experiments, rabbits were maintained on a fixed diet for over 1 week till their body weights were stabilized. Thereafter blood was collected, and the normal serum fat components in each group were measured.

Fig. 9 Distribution graph of total cholesterol, ester cholesterol, phospholipid value in blood (normal 36 rabbits).

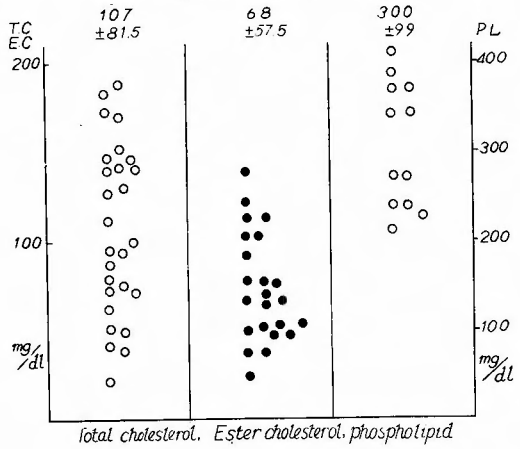


Fig. 10 Changes in blood cholestrol levels following repeated intravenous infusion of 7% glucose solution.

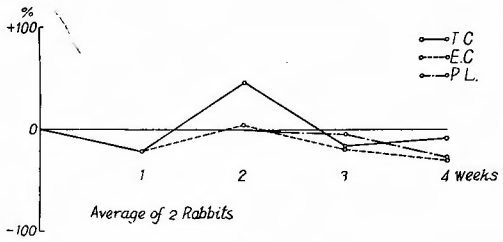


Table 8 Changes in blood cholesterol (total cholesterol, ester cholesterol and phospholipid) levels following repeated intravenous infusion of 7% glucose solution (mg/dl).

	Total chol.		Ester chol.		Phospholipid		EQ
Before Injection	135	%	83	%		%	61.4
1 Week	106	−22	65	−22			61.3
2 Week	199	+47	87	+ 4	320		43.7
3 Week	113	−17	68	−19	317	− 1	60.2
4 Week	125	− 8	58	−31	230	−29	46.4

ii) Results of the Injection of a 7% Glucose Solution (Control Experiments)

As a control for the experiments described later, a 7% glucose solution (all the sesame oil emulsions contain glucose in a concentration of 7%) was infused into the ear vein of the rabbits. Daily infusion of 2.5 cc per kg body weight was repeated for 4 weeks and changes in the serum concentration of T-chol., E-chol. and PL were followed. The results, as shown in Table 8. and Fig. 10, showed only fluctuations within the physiological range.

iii) Changes in the Serum Concentration of T-chol., E-chol. and PL Observed Following the Infusion of the 20% Sesame Oil Emulsion Containing Peroxides (Peroxide Value 5～10) and Cholesterol in Various Concentrations

Various kinds of the 20% sesame oil emulsion containing peroxides of values 5～10 and cholesterol in concentrations of 0%, 0.1%, 0.3%,...0.7% and 1.0% were prepared. Each of the above was infused repeatedly for 4 weeks. The results are shown in Fig. 11; those infused with emulsions containing no cholesterol or cholesterol in concentrations of 0.1% and 0.3% showed no significant change in the concentrations of serum T-chol., E-chol. and PL, the daily fluctuations being within the physiological range just as were those of the control experiments. However, in the animals infused with emulsions containing cholesterol in concentrations over 0.5% the serum concentration of T-chol. showed a distinct tendency to rise day by day.

iv) Measures to Prevent Peroxide Toxicity in Connection with Cholesterol

Fig. 11 Changes in blood cholesterol levels following repeated intravenous administration of sesame oil emulsion (peroxide value: 5~10).

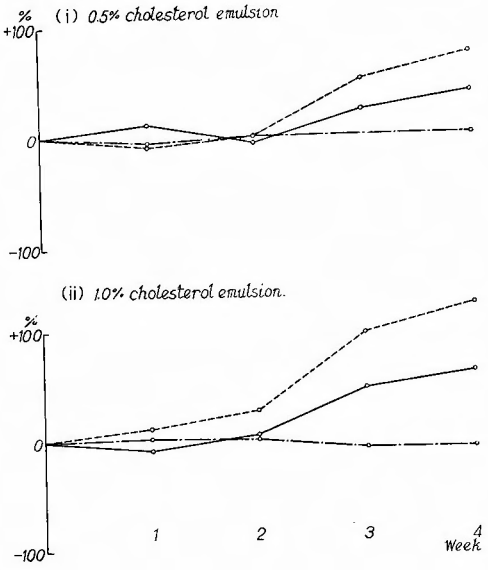
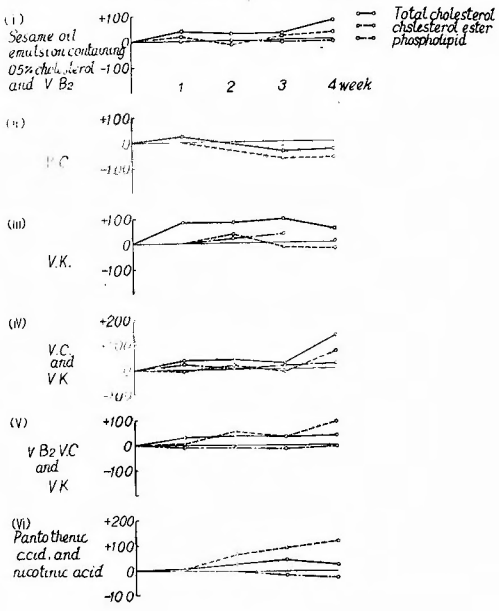


Fig. 12 Weekly changes of blood cholesterol following repeated intravenous administration of sesame oil emulsion containing cholesterol in concentration of 0.5% and various vitamins.



Metabolism

The 20% sesame oil emulsion containing cholesterol in a concentration of 0.5%, peroxides, and various vitamins was infused for 4 weeks and changes in the concentration of serum T-chol., E-chol. and PL were followed daily. In this connection, Osa and Hashino of our laboratory have demonstrated the infused fats are oxidized smoothly and completely, only when various vitamins are infused simultaneously with the fat emulsion. Myasnikow has reported that vitamin C acts to check the development of atherosclerosis in rabbits fed a diet containing cholesterol, while vitamin A, vitamin B_1 and vitamin B_2 have little effect. Meanwhile, Olsen et al. have pointed out that deficiency of vitamin B_6 may cause hypertension in rats. Reinhart and Greenberg have reported the same finding in monkeys. Altschul has shown that nicotinic acid is effective in reducing the cholesterol concentration in rabbit serum.

Therefore, vitamin B_1, B_2, B_6, C, K, nicotinic acid and pantothenic acid were used with the fat emulsion, separately or in various combinations, and changes occurring in the concentration of serum T-chol., E-chol. and PL in rabbits were observed. As indicated in Fig. 12, vitamin B_2 and vitamin C particularly were proved the most effective in checking the increase in the concentration of T-chol. and the disturbance in the esterification of cholesterol caused by the peroxide infusions. Other vitamins were found to have little effect.

It has long been known that yeast and vitamin B_2 are effective in neutralizing

Table 9 Changes in fat content in various organs following repeated intravenous administration of sesame oil emulsion and various vitamins (4 weeks).

Infused substances	Lung	Liver		Spleen
		Kupffer cells	Liver cells	
7% glucose solution	−	−	−	−
sesame oil emulsion (S. O. E) + vitamin B_2 + vitamin C	±	+	−	++
S.O.E. containing 0.1% cholesterol	±	+	−	+
S.O.E. containing 0.3% cholesterol	±	+	−	+
S.O.E. containing 0.5% cholesterol	+	+	−	+
S.O.E. containing 0.5% cholesterol + V. B_2	+	++	−	+
S.O.E. containing 0.5% cholesterol + V. C	+	++	−	+
S.O.E. containing 0.5% cholesterol + V. K	+	++	−	+
S.O.E. containing 0.5% cholesterol + Pantothenic acid + Nicotinic acid	+	±	−	±
S.O.E containing 0.5% cholesterol + V. B_1 + V. B_2 + V. C	−	+	−	+

the toxicity of fish oil. The toxicity of fish oil is attributed not merely to its large content of highly unsaturated fatty acids but also to the fact that fish oil is likely to contain a larger amount of peroxides than other fats, because highly unsaturated fatty acids most easily undergo auto-oxidation. The use of vitamin B_2 to neutralize the toxicity of fish oil may be interpreted in essence as an application of the preventive procedures explained in the above experiments.

Vitamin B_1 and a glucose solution, which have been mainly used for improvement of liver function, were also added to the emulsion in order to augment the effect of vitamin B_2 and C. As indicated in Fig. 13, even after repeated infusions with the emulsion containing peroxides for 8 weeks, there was recognized neither abnormal increase in serum T-chol., nor disturbance in the esterification of cholesterol, and the rabbits maintained a satisfactory condition throughout the observation periods.

Fig. 13 Changes in blood cholesterol levels following repeated intravenous administration of sesame oil emulsion containing cholesterol in concentration of 0.5%, vitamin B_1, vitamin B_2, vitamin C, and 20% glucose solution.

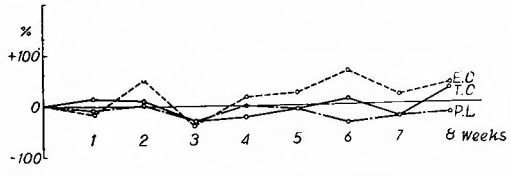
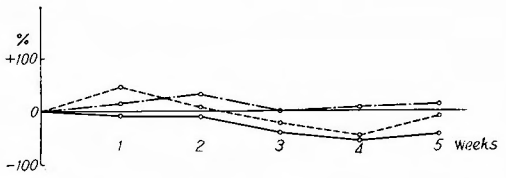


Fig. 14 Changes in blood cholesterol levels following repeated intravenous administration of sesame oil emulsion containing cholesterol in concentration of 0.5%, vitamin B_1, vitamin B_2, vitamin C, 20% glucose solution and lecithin.



Moreover, when oral lecithin was added to the above medications, serum T-chol. even showed a tendency to decrease (Fig. 14).

2) Results of Histological Examinations

i) Reaction of the Various Organs to the Repeated Infusion of the 20% Sesame Oil Emulsion Containing Different Amounts of Cholesterol but Free of Peroxides

It has been well recognized that fatty liver and vascular lesions can be experimentally produced by feeding animals diets containing a large amount of fat (30～40%) and cholesterol (1～2%).

In the present experiments, each of the 20% sesame oil emulsions containing cholesterol in different concentrations was intravenously infused into the rabbits, daily for 4 weeks. Rabbits were sacrificed by bleeding after the last infusion, and their organs were removed immediately and investigated histochemically by Oil-red-O staining. According to our colleagues, ASADA, IZUKURA and SHIROTANI, the primary action on chylomicra absorbed into the blood stream takes place in the lung, liver and spleen; therefore these three organs were examined. The results are shown in Table 9. The amount of lipid phagocytized by the alveolar phagocytes, KUPFFER'S cells of the liver and the reticuloendothelial cells of the spleen following infusion of the emulsion containing cholesterol in a concentration of 0.5% was the same as the amount in the cases receiving a cholesterol free emulsion. The hepatic cells were quite intact in both groups. It may be postulated from these results that infused cholesterol, in limited daily doses can be smoothly treated and utilized in the body without accumulation in the lung, liver or spleen.

Table 10 Changes in fat content in various organs following repeated intravenous administration of sesame oil emulsion containing cholesterol in concentration of 0.7～1.0% and various vitamins (4 weeks).

Infused substances	Lung	Liver		Spleen
		Kupffer cells	Liver cells	
Sesame oil emulsion containing 0.7% cholesterol	++	+	−	+
S.O.E. containing 0.7% cholesterol + vitamin B_2 + vitamin C	++	++	−	++
S.O.E. containing 0.7% cholesterol + vitamin B_6	+++	+++	±	+++
S.O.E. containing 0.7% cholesterol + vitamin B_2 + vitamin B_6	+++	+++	±	+++
S.O.E. containing 0.7% cholesterol + vitamin B_2 + vitamin K		++	−	++
S.O.E. containing 1.0% cholesterol	+++	+++	±	+++
S.O.E. containing 1.0% cholesterol + vitamin B_2 + vitamin C	+++	++～+++	±	++

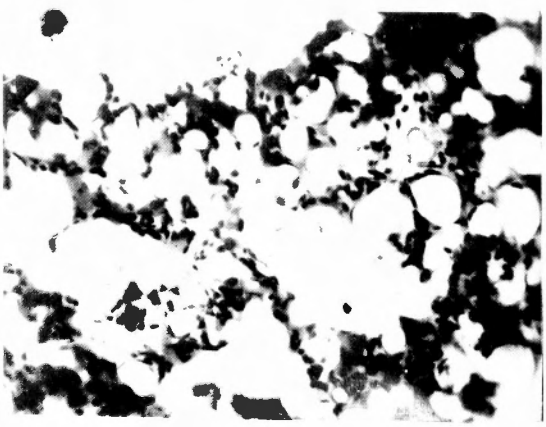


Fig. 15 Fat in lung after repeated intravenous administration of sesame oil emulsion containing cholesterol in concentration of 1.0% for 4 weeks. ×200, Oil-red O staining.

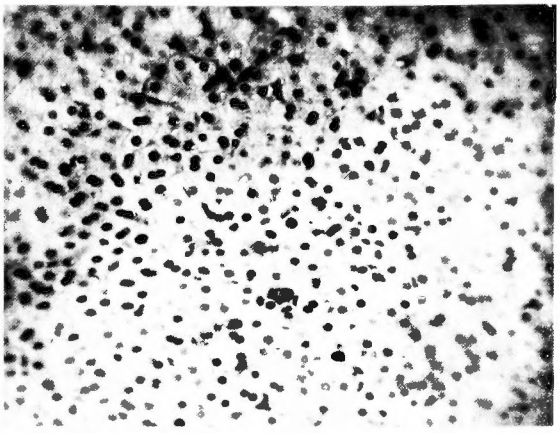


Fig. 16 Fat in liver after repeated intravenous administration of sesame oil emulsion containing cholesterol in concentratration of 1.0% for 4 weeks. ×200, Oil-red O staining.

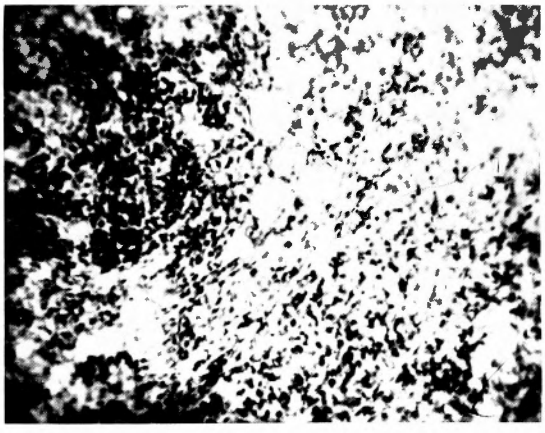


Fig. 17 Fat in spleen after repeated intravenous administration of sesame oil emulsion containing cholesterol in concentration of 1.0% for 4 weeks. ×50, Oil-red O staining.

However, in the cases infused with emulsions containing cholesterol in concentrations over 0.7%, especially 1.0%, much larger amounts of lipids were found in the reticuloendothelial cells of these three organs (Table 10). Moreover, even in the hepatic parenchymatous cells, lipid infiltrations were recognized, though they were not clear. The general results of these histochemical findings completely agree with the results of the biochemical experiments on cholesterol in serum (Figs. 15, 16, and 17). Both lead us to the conclusion that 0.5% is the maximum concentration of cholesterol which can be safely infused in combination with 2.5 cc of the 20% sesame oil emulsion per kg of body weight without causing any disturbance in the lipid metabolism of rabbits.

Findings in the lungs were of great interest. Following infusion of the emulsion containing 1.0% cholesterol, lipid positive to Oil-red-O staining was found not only in the phagocytes of the lung, but also densely in the alveolar cavities. This lipid was believed to be mainly cholesterol, because in the cases receiving infusions of cholesterol free emulsion, no lipid was found in spite of repeated infusions for 4 weeks. It is difficult to explain these findings unless we postulate a cholesterol excretory function of the lungs. ASADA of our laboratory has demonstrated that the lungs can excrete glycerides. ABELOUS, REMOND, COLOMKIES and BERNARDBERG also have postulated that the lungs play a role in cholesterol metabolism from the fact that blood in the right

heart contains a higher concentration of cholesterol than that in the left. The above mentioned histochemical findings obtained by the author are clear proofs for such a postulation. That is, when hypercholesterolemia continues for a long time, the lung excretes the surplus cholesterol into the alveolar cavities; accordingly it acts as a regulator for maintaining a normal serum cholesterol concentration. SEEMAN has also expressed the same opinion. He infused cholesterol into the ear veins of rabbits, to a total of 12～14g over one month, observed cholesterol mass in the pulmonary vessels and hyperplasia of the alveolar walls and of the alveolar septa, and reached the conclusion that lung had a cholesterol excretory function.

Table 11 shows the results in cases infused with the sesame oil emulsions containing cholesterol in a concentration of 0.5% in combination with vitamin B_1, B_2, and C daily for 8 weeks. The results were nearly the same as in the cases infused with the cholesterol free emulsion for 4 weeks. The liver, spleen and lungs were quite free of lipid accumulation.

ii) Reaction of the Organs to the Intravenous Infusion of the 20% Sesame Oil Emulsion Mixed with Peroxides of Value 5～10 and Containing Various Amounts of Cholesterol

20% sesame oil emulsions containing peroxides and cholesterol in concentrations of 0.5% and 0.7% were prepared. This solution similarly combined with vitamins was infused intravenously daily for 4 weeks. The following results were obtained.

The amount of lipid in the lung, liver and spleen was greater than that found in the cases receiving infusions of peroxide free emulsions. However, those receiving vitamin B_2 and C in the emlusion showed the least amount of lipid of this group of cases. That is, the results of these histochemical experiments were identical with those of the above mentioned biochemical experiments on the serum lipid components. The merits of using vitamin B_2 and C in the emulsion are thus verified by these results.

iii) Specific Function of Cholesterol

It has been estimated that about 50% of orally introduced cholesterol is esterized before absorption; that is, the absorption of cholesterol necessitates its esterification and the action of bile acids in the intestine. Moreover, when some glycerides or fatty acids are introduced with cholesterol they accelerate the absorption of the latter.

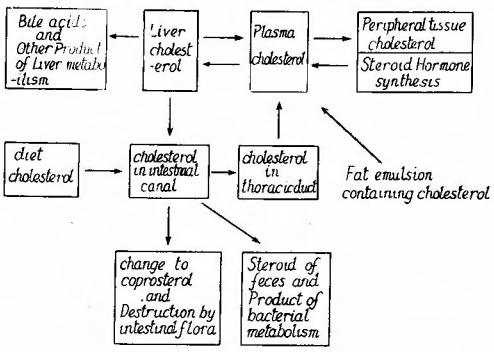
A part of the cholesterol, about 2.0g daily, is synthesized within the body, the liver being the main producer.

Table 11 Changes in fat content in various organs following repeated intravenous administration of sesame oil emulsion containing cholesterol in concentration of 0.5%, vitamin B_1, vitamin B_2, vitamin C and glucose for 8 weeks.

Infused substances	Lung	Liver		Spleen
		Kupffer cells	Liver cells	
Sesame oil emulsion containing 0.5% cholesterol + vitamin B_1 + vitamin B_2 + vitamin C + glucose	−	+	−	−

Part of the cholesterol serves as material for steroid hormones and bile acids and as a constituent of tissue cells. Another portion passes through the liver to be excreted into the intestine. The outline of a probable process of cholesterol metabolism is shown in Fig. 18.

Fig. 18 Cholesterol metabolism



It is a matter of course to assume a hepatointestinal circulation of cholesterol. Although the surplus cholestrol is excreted into the intestines by this circulation, a part of it is reabsorbed into the thoracic duct. The physiological significance of cholesterol as such has not been clarified yet.

A simple lipid is a glycerin ester of fatty acids and is insoluble in water because the combination of hydroxy groups of glycerin and carboxyl groups of fatty acid closes its polar groups. By adding a compound, which has both polar and non-polar groups in one molecule, a simple lipid disperse in water very well and becomes an emulsion. This phenomenon can be explained in the following manner: a protein, a molecule of which has both polar and non-polar groups, lowers the surface tension by combining with a lipid molecule by its non-polar group on the one hand, and with a water molecule by its polar group on the other. Since lecithin and cholesterol have the same effect as protein, one of the physiological roles of cholesterol is to convert a simple glyceride into an emulsified state.

ASADA in our laboratory has noticed that when cod liver oil emulsions are repeatedly infused intravenously in experimental animals, a pigment exhibiting a brown or greenish brown color when stained with hematoxylin eosin is deposited in the spleen. Our colleague OTANI has found out that even after repeated intravenous infusions of sesame oil emulsion the animal spleen shows the same pigment deposition, but far less than that found after infusions of cod liver oil emulsion. And he has identified this pigment as hemosiderin. It has been demonstrated that glycerides containing lower fatty acids and highly unsaturated fatty acids cause a more marked pigment deposition than those consisting only of the higher saturated fatty acids, oleic, linoleic and linolenic acid. This is one of the reasons why we have advocated the use of glycerides such as sesame oil consisting only of the higher saturated fatty acids, oleic, linoleic and linolenic acid. Although we are using only sesame oil emulsion for intravenous infusion of fat in clinical practice, even the sesame oil emulsion is not completely safe from causing hemolysis, consequently some counter measures against such hemolysis are necessary. In the course of the experimental work we have found that cholesterol has a marked antihemolytic action. To verify this, the 20% sesame oil emulsion containing cholesterol in various concentrations was infused intravenously in rabbits, alone or in combination with various vitamins, repeatedly for 4 weeks. Thereafter the animals were sacrificed by bleeding and the degree of hemosiderin deposition in their spleens was compared with

that in the spleens of the animals infused with 2.5 cc of a 7% glucose solution per kg daily for 4 weeks. The results shown in Table 12 reveal that in adding cholesterol to the 20% sesame oil emulsion, a concentration of 0.1% is not sufficient, but 0.3% is necessary to be an effective antihemolytic agent and the addition of more than 0.3% does not cause any recognizable difference in the degree of hemosiderin deposition.

Table 12 Changes in hemosiderin content in spleen following repeated intravenous administration of sesame oil emulsion containing cholesterol and various vitamins for 4 weeks.

Infused substances	Changes in hemosiderin content(spleen)
Sesame oil emulsion + vitamin B_2 + vitamin C	+2
Sesame oil emulsion containing 0.1% cholesterol	+1～−1
Sesame oil emulsion containing 0.3% cholesterol	−2
Sesame oil emulsion containing 0.5% cholesterol	−2
Sesame oil emulsion containing 0.7% cholesterol	0～−2
Sesame oil emulsion containing 1.0% cholesterol	−1～−2
Sesame oil emulsion containing 0.5% cholesterol + B_2	0～−1
Sesame oil emulsion containing 0.5% cholesterol + C	0～−2
Sesame oil emulsion containing 0.5% cholesterol + K	0～−1
Sesame oil emulsion containing 0.5% cholesterol + Pantothenic acid + Nicotinic acid	+1～0
Sesame oil emulsion containing 0.5% cholesterol + B_1 + B_2 + C	0～−1
Sesame oil emulsion containing 0.7% cholesterol + B_2 + C	0
Sesame oil emulsion containing 0.7% cholesterol + B_6	0～−1
Sesame oil emulsion containing 0.7% cholesterol + B_2 + B_6	0～−1
Sesame oil emulsion containing 0.7% cholesterol + B_2 + K	+1～−1
Sesame oil emulsion containing 1.0% cholesterol + B_2 + C	0
Sesame oil emulsion containing 0.5% cholestesol + B_1 + B_2 + C	0
Sesame oil emulsion containing 0.5% cholesterol + B_1 + B_2 + C (8 weeks)	+1～−2

In considering the antihemolytic action of cholesterol, it is of very important physiological significance that cholesterol is always present in the intestines due to the hepatointestinal circulation of cholesterol, even when no cholesterol is introduced by mouth. When orally introduced glycerides are absorbed and enter the blood stream in the form of chylomicra, cholesterol in the intestines is absorbed at the same time and acts to prevent the hemolytic action of glycerides. It is highly probable that this phenomenon is one of the physiological roles of the hepatointestinal circulation of cholesterol.

V. SUMMARY

Our laboratory has made an effort to clarify fat metabolism in vivo by preparing emulsions of natural oils (mixed glycerides) or artificial simple glycerides and infusing them intravenously into various animals. The following results have been obtained. The lower fatty acids, highly unsaturated fatty acids, docosenoic acid, eicosenoic acid etc. are shifted only to the parenchymatous cells of the liver in the form

of phospholipids (lipoprotein) and undergo further metabolism, and they are the ones which undergo, for the most part, so-called indirect oxidation. Whereas higher saturated fatty acids, oleic acid, essential fatty acids etc. are shifted in the form of phospholipids (lipoprotein) not only to the parenchymatous cells of the liver but also directly to the extrahepatic tissues and undergo further metabolism, and they are the ones which, for the most part, undergo so-called direct oxidation. Therefore, the administration of fatty acids of the former group in large quantities seems to impose a heavy burden on the liver and marked ketone body production. However, the administration of fatty acids of the latter group seems to be ideal since the burden on the liver is not heavy. This is in agreement with the results gained by MATSUDA, SATOMURA and others of our laboratory investigating liver function, sensitivity to alloxan, glycogen amount in the liver, blood sugar levels and the growth curve of experimental animals. It follows, therefore, that the glycerides containing the fatty acids which undergo, for the most part, so-called indirect oxidation should be avoided as much as possible, and the glycerides consisting of fatty acids which, for the most part, undergo so-called direct oxidation is recommended for administration as a nutrient, provided that they contain enough essential fatty acids such as linoleic and linolenic acid.

Moreover, care must be taken in the preservation and cooking of the oils to avoid contamination with peroxides. For it has already been proved by our experiments that feeding the animals with a diet containing a high percentage of peroxides and of fatty acids such as highly unsaturated fatty acids, consistently causes fatty liver.

The present experiment has examined the extent of the functional disturbance of the liver caused by peroxides from the standpoint of cholesterol metabolism, and has clarified that the introduction of peroxides is very injurious to the liver even in normal animals. It has been verified that the use of vitamin B_2 and vitamin C acts to check the toxicity of peroxides, and that a glucose solution and vitamin B_1, which have been applied to protect the liver function, act, if used in a sufficient dose, to increase the effectiveness of vitamin B_2 and C. It is pointed out, therefore, that the introduction of peroxides is particularly dangerous to patients with vitamin deficiencies, with functional disturbances of the liver and under various stresses.

This knowledge indicates that the administration of fat is a very desirable treatment for many pathological conditions. KUYAMA, TATSUMI and HANAFUSA of our laboratory have found that the use of the sesame oil emulsion causes a remarkable protein sparing effect. MATSUDA has shown that it has beneficial effects on carbohydrate metabolism. TAMAKI and KOBAYASHI have noted that its use before and after surgical operation has favorable effects on fluid metabolism and that the administration of fat emulsion is absolutely necessary for the maintenance of the plasma colloidal osmotic pressure. And NAGASE has shown that a deficiency of the essential fatty acids is a probable cause of capillary bleeding and edema formation.

KISHIMOTO has examined the peroxide value of the sesame oil used for cooking, refined sesame oil and sesame oil of the J. P. IV. specification, which are generally

used in determing the nutritional effect of fat, and shown that these oils contain a considerable amount of peroxides, and that the peroxide value is probably increased by cooking. TOBE's paper chromatographic analysis of the same sesame oils has revealed that these three types of sesame oil differ greatly in their fatty acid composition. Consequently, it is very important to examine the qualitative properties of the fats prior to their administration to experimental animals.

Furthermore, it has been shown, by measuring the degree of hemosiderin deposition in the spleen after the administration of the 20% sesame oil emulsion containing cholesterol at various concentrations, that cholesterol counteracts the hemolysis caused by glycerides and fatty acids. The author believes that its anti-hemolytic action is probably one of the reasons for the hepatointestinal circulation of cholesterol. For it is most probable that cholesterol always exists in the intestines in a constant amount and is absorbed into the blood stream to check the hemolysis caused by glycerides and fatty acids. This is the most logical explanation for the fact that the presence of glycerides and fatty acids accelerates the absorption of cholesterol through the intestinal mucous membrane. When a prolonged hypercholesterolemia is produced in experimental animals by the administration of emulsions containing cholesterol, the lung excretes the surplus cholesterol into the alveolar cavities and plays a role in regulating serum cholesterol concentration. It is very interesting that the lung as well as the liver plays a role in controlling the serum cholesterol concentration.

VI. CONCLUSION

To sum up, in the present experiments, two groups of the fat emulsion were mainly used. One group was the 20% sesame oil emulsion containing cholesterol in various quantities but free of peroxides. The other was the 20% sesame oil emulsion containing both cholesterol and peroxides. By applying these emulsions the author has come to the following conclusions.

1) Consideration of the qualitative properties of the fats is prerequisite to the examination of their nutritional effects.

2) The peroxides produced by auto-oxidation of fat are very injurious to the body, especially to the liver. Hence, cooking and preservation of fat must be performed with proper precautions not to include peroxides.

3) In checking the toxicity of peroxides, vitamin B_2 and C are effective to some extents. And it is recommended that an adequate amount of glucose and vitamin B_1 be combined with them. The toxicity of the peroxides is more marked in patients with functional disturbances of the liver, with vitamin deficiency, and under stress.

4) Cholesterol acts to check the hemolysis caused by glycerides or by fatty acids. This fact offers a partial explanation for the significance of the hepatointestinal circulation of cholesterol.

5) The lungs, having a cholesterol excretory function, take part in controlling the amount of cholesterol in the blood.

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和 文 抄 録

栄養学的効果に及ぼす脂質の質的組成の影響に関する実験的並びに臨床的研究

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過酸化物混入の怖れはなく，且つ種々の割合にCholesterol を含有した20%ゴマ油乳剤と更に過酸化物を混入して，而もなお種々の割合にCholesterolを含有した20%ゴマ油乳剤を特別に調製し，これを臨床的並びに動物実験に応用することによつて次の結論に到達した．

(1) 脂質の栄養学的効果並びに弊害等を論ずるに当つては，まず投与脂質の質的組成に対する充分な配慮が払われなければならない．

(2) 脂質の自働酸化によつて生ずる過酸化物は生体一殊に肝臓に対して極めて有害的に作用する．従つて過酸化物混入の怖れのある脂質の摂取は極力これを避け，又脂質の保存，調理にも今後充分な配慮が必要である．

(3) 過酸化物の毒性防止にはビタミンB_2及びCの投与はある程度迄効果を期待し得るが，更に出来れば之等に加うるに充分な糖及びビタミンB_1の投与が行われなければならない．従つて肝機能障害，ビタミン欠乏症，各種Stress 状態下にあるものに於てはその毒性が益々助長される怖れがある．

(4) Cholesterol はグリセライドあるいは脂酸にもとづく溶血作用に対して防止的に作用するが，Cholesterolの肝腸循環の行われている理由の一つもこの点にあるのかも知れない．

(5)肺臓も亦 Cholesterol の排泄機能を有し，血中Cholesterol 含有量の調節に関与しているものと思われる．